The Missing Piece: Recent Approaches Investigating the Antimicrobial Mode of Action of Essential Oils

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ABSTRACT: Antibiotic resistance is a major global health issue that has seen alarming rates of increase in all parts of the world over the past two decades. The surge in antibiotic resistance has resulted in longer hospital stays, higher medical costs, and elevated mortality rates. Constant attempts have been made to discover newer and more effective antimicrobials to reduce the severity of antibiotic resistance. Plant secondary metabolites, such as essential oils, have been the major focus due to their complexity and bioactive nature. However, the underlying mechanism of their antimicrobial effect remains largely unknown. Understanding the antimicrobial mode of action of essential oils is crucial in developing potential strategies for the use of essential oils in a clinical setting. Recent advances in genomics and proteomics have enhanced our understanding of the antimicrobial mode of action of essential oils. We might well be at the dawn of completing a mystery on how essential oils carry out their antimicrobial activities. Therefore, an overview of essential oils with regard to their antimicrobial activities and mode of action is discussed in this review. Recent approaches used in identifying the antimicrobial mode of action of essential oils, specifically from the perspective of genomics and proteomics, are also synthesized. Based on the information gathered from this review, we offer recommendations for future strategies and prospects for the study of essential oils and their function as antimicrobials.

KEYWORDS: Essential oil, genomic analysis, mode of action, proteomic analysis

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Introduction

Antibiotics have been used to treat bacterial infections for almost a century since the discovery of penicillin in 1928 by Alexander Fleming. The commercialization of penicillin in 1944 lowered the mortality statistics for those who have contracted bacterial infections. Over the years, diverse groups of antibiotics from different sources have been discovered, characterized, and applied in a clinical setting to offset antibiotic resistance. However, newer resistance mechanisms have consistently emerged almost in tandem with the discovery of a novel antimicrobial. In recent years, the rate of novel antimicrobial discovery has substantially decreased. This decline can be attributed to the failure of current antibiotic discovery platforms in discovering novel high-efficacy antibiotics.[1] Apart from this, new antibiotics are subjected to a complicated assessment of their overall toxicity and side effects, ranging from cell-line to animal studies and resulting in human clinical trials; all of these without the commercialization process. The combination of high investments, relatively short effective duration (due to resistance) and relatively low price compared to other drugs has made the development of antibiotics unappealing for the pharmaceutical industry.[2]

Over the years, studies have been performed on plant secondary metabolites such as essential oils in the hope of discovering an alternative that can alleviate the current challenge of antibiotic resistance.[3-6] Essential oil is a concentrated plant secondary metabolite composed of a mixture of chemical compounds ranging from terpenes and terpenoids to aromatic compounds that gives fragrance to a plant. Numerous studies have established the antimicrobial potential of essential oils against bacteria, fungi, and viruses.[4,6-8] Most essential oils have been found to produce antibiotic effects by acting on the bacterial membrane and the efflux system.[9-11] However, specific mechanisms for disrupting the bacterial membrane and inhibiting efflux remain largely inconclusive.

This review paper consolidates past and present knowledge on essential oil research with an emphasis on antimicrobial activity, in addition to reviewing existing approaches for investigating the mode of action of essential oils. This will be followed by a discussion on the latest approaches for bridging the missing piece of previous research. The limitations of essential oil research and prospects will also be discussed.

Past Essential Oil Studies

Antimicrobial activity

Over the years, researchers have demonstrated the antimicrobial activity of various essential oils against a wide range of microorganisms. Most essential oils studied are derived from plants that are commonly used in our daily lives, such as those used in the aromatherapy, food and beverage industries, and cosmetics industries. Examples include lavender, eucalyptus, thyme, peppermint, and cinnamon bark essential oils. The antimicrobial potency of various essential oils is the main determinant of their

potential use in clinical applications. Some of the major antibacterial, antifungal, and antiviral potentials of essential oils have therefore been collected in the sections below.

Antibacterial activity. The Kirby Bauer and broth microdilution assays are two of the most common methods used to evaluate the minimum inhibitory activity of essential oils. The Kirby Bauer assay uses disks infused with a known amount of potential antimicrobial to detect the bacterial susceptibility by measuring the inhibition zone produced. Broth microdilution, on the other hand, infuses varying concentrations of potential antimicrobial in the microculture while detecting the bacterial viability using resazurin, allowing a minimum inhibitory concentration (MIC) to be determined.[9,11] The following section summarizes a few recent examples of essential oils and their antibacterial activity.

The lavender essential oil has been screened for their antibacterial use by numerous research groups; results indicate that lavender-based essential oil is bioactive against a wide range of bacteria.[5,11,12] Yang et al[5] demonstrated the antibacterial activity of lavender essential oil against clinically-relevant strains of *Klebsiella pneumoniae* with a MIC of 10% (v/v). Another study by Hossain et al[12] showed that lavender oil is bactericidal against pet turtle-isolated pathogenic bacteria, including *Aeromonas spp, Citrobacter freundii, Proteus mirabilis, Salmonella enterica*, and *Pseudomonas aeruginosa*, with a MIC of 0.5% to 2% (v/v). Cinnamon bark essential oil also demonstrated antibacterial activity against antibiotic-resistant strains of *Escherichia coli* with a MIC of 0.009% to 0.078% (v/v) as shown by Yap et al[4] Besides, Firmino et al[13] showed the antibacterial activity of cinnamon bark essential oil against *Staphylococcus aureus, S. epidermis, Streptococcus pyogenes, P. aeruginosa*, and *E. coli* have MIC values of 0.25% to 0.5% (v/v) of MIC. In a similar study by Liang et al,[14] peppermint essential oil is found to be effective against *Listeria monocytogenes* and *S. aureus* at a MIC of 0.5% (v/v). Similarly, Saeed et al[15] showed that peppermint essential oil is inhibitory against a panel of *Enterobacteriaceae* with a MIC of 10% (v/v). Table 1 summarizes the reported antibacterial activity of essential oil.

Antifungal activity. As with antibacterial activity, the Kirby Bauer and broth microdilution assays are usually the methods employed. However, the Kirby Bauer assay is the more preferred method because fungal species do not grow homogeneously in liquid culture, which will interfere with the determination of the MIC.[16] The following section consolidates a few recent cases of essential oils and their antifungal activity.

Hammer et al[16] demonstrated the antifungal activities of tea tree oil against *Candida albicans, C. glabrata*, and *Saccharomyces cerevisiae* with MICs ranging between 0.5% and 1% (v/v). Recently, Powers et al screened a total of 60 commercially available essential oils, such as cinnamon bark essential oil, lemon essential oil, peppermint essential oil, and others, against *Aspergillus niger, C. albicans*, and *C. neoformans.* Based on the study, the MIC of essential oils ranged from 0.02% to 1.250% (v/v).[17] On the other hand, Hu et al[18] published the antifungal activities of cinnamon bark, peppermint, citronella, anise, pepper, and clove essential oil against *A. niger, A. oryzae*, and *A. ochraceus* with MICs from 0.06% to 0.2% (v/v). Table 1 presents the reported antifungal activities of some essential oils.

Antiviral activity. Plaque reduction assay is used to determine the antiviral activity of essential oil. The plaque reduction assay uses a cell culture that serves as the host for the target virus. The target virus is first cultured in cells followed by treatment with potential antivirals. Plaque-forming units are later determined to enumerate the minimum amount required to reduce plaque-forming units by 100%. The following section consolidates a few recent examples of essential oils and their antiviral activity.

Essential oils from ginger, thyme, hyssop, and sandalwood have found to be bioactive against herpes simplex virus.[19] In addition, Brochot et al[6] demonstrated that essential oils from cinnamon bark, eucalyptus, carrot, and rosemary are effective against viral particles of influenza A virus H1N1 and oral herpes simplex virus HSV1, in which plaque reduction of more than 99% was detected using 1% (v/v) essential oil. Vuko et al[20] also demonstrated the antiviral activity of *Micromeria croatica* essential oil against the cucumber mosaic virus. Table 1 summarizes the reported antiviral activity of essential oils.

Mode of action

Antimicrobial activities of essential oils have been widely reported. However, the mode of action of these essential oils is still unclear. Traditionally, the mode of action of essential oils was inferred mainly based on biochemical assays. Due to technical limitations, these assays are generally poor in the identification of exact causes or pathways leading to antimicrobial effects. Nonetheless, an essential oil is believed to have a structural impact on the bacterial membrane and its transport system.

Membrane disruption. Bacterial membrane plays an important role in the regulation of cellular osmotic pressure and the influx and efflux of biomolecules. Thus, a compromised membrane will disrupt osmotic pressure, leading to intracellular leakage and eventually destroy the cell. One of the main postulated modes of action of essential oil is its ability to disrupt bacterial membranes. In their work on the bactericidal effect of tea tree oil against *E. coli* and *S. aureus*, Cox et al demonstrated that cells treated with tea tree oil have higher fluorescence under propidium iodide (PI) staining, and increased intracellular material leakage and potassium ion efflux. All of these indicated a potential disruption of the bacterial membrane.[34] It is noteworthy that PI stains the genetic materials of the cell membrane of the compromised bacterial cells whereas the intracellular material leakage and potassium ion efflux assay detect the leakage of

Table 1. Antimicrobial activity of essential oils.

ESSENTIAL OIL	ANTIBACTERIAL		ANTIFUNGAL		ANTIVIRAL	
Cinnamon bark	*E. coli* *K. pneumoniae* *S. aureus* *S. epidermis* *S. pyogenes* *P. aeruginosa*	4,9,13	*A. niger* *C. albicans* *C. neoformans*	17,18	Influenza A virus H1N1 Oral herpes simplex virus HSV1	6
Eucalyptus	*E. coli* *Edwardsiella tarda* *Lactococcus garviae* *S. iniae* *S. parauberis* *S. aureus*	21-23	*Fusarium* sp. *Aspergillus* sp. *Ulocladium* sp. *Coprinellus* sp. *Penicillium* sp.	24,25	Influenza A virus H1N1 Oral herpes simplex virus HSV1	6
Lavender	*Aeromonas spp* *C. freundii* *K. pneumoniae* *P. mirabilis* *P. aeruginosa* *S. enterica*	5,12	*Alternaria alternate* *A. fumigatus* *C. albicans* *Chaetomium globosum* *Cladosporium cladosporoides* *P. chrysogenum*	26-28	-	-
Peppermint	*L. monocytogenes* *S. aureus* *Enterobacteriaceae*	15,18	*A. niger* *A. oryzae* *A. ochraceus* *C. albicans* *C. neoformans*	17,18	Oral herpes simplex virus HSV1 Oral herpes simplex virus HSV2	29
Rosemary	*E. coli* *Enterococcus faecalis* *S. aureus*	30-32	*A. flavus* *C. albicans*	30,33	Influenza A virus H1 N1 Oral herpes simplex virus HSV1	6
Tea tree	*E. coli* *Propionibacterium acnes* *S. aureus*	16,34,35	*C. albicans* *C. glabrata* *S. cerevisiae* *Trichophyton rubrum* *T. mentagrophytes*	16,36	Oral herpes simplex virus HSV1 Oral herpes simplex virus HSV2 Influenza A virus H1 N1	8,37

Abbreviation: HSV, herpes simplex virus.

intracellular materials, such as genetic materials, proteins, and potassium ions, in the event of membrane disruption. Moreover, Yap et al[11] investigated the antimicrobial activity and mode of action of lavender essential oil against antibiotic-resistant *E. coli*. Their study showed that lavender essential oil affects the membrane zeta potential of *E. coli* cells after exposure. Membrane zeta potential reveals the surface charge of the bacterial membrane and a change in the value is associated with membrane disruption. Furthermore, the permeability of the cell membrane has also increased, as shown in the outer membrane permeability assay using sodium dodecyl sulfate (SDS) as a membrane-disrupting probe. Their study was validated by the microscopic analysis of the *E. coli* treated with lavender essential oil, in which the disruption of cell structure and morphology was visualized.[11] Consistent with these findings, Zhang et al[38] demonstrated the membrane disruption ability of black pepper essential oil against *E. coli*. The study reported changes in the membrane potential of *E. coli* cells following treatment with black pepper essential oil. The intracellular leakage was also observed in the cells following treatment with black pepper oil and the cell membrane disruption was further validated via scanning electron microscopy.[38] Subsequently, Yang et al showed that cinnamon essential oil has exerted their antimicrobial ability against multidrug-resistant *K. pneumoniae* by disrupting their bacterial membrane. The membrane disruption was assessed by measurement of zeta potential, outer membrane permeability and scanning electron microscopy.[9]

Efflux inhibition. Another postulated mode of action of essential oil is its ability to inhibit the bacterial efflux system. The bacterial efflux system comprises of specialized channel proteins located on the bacterial membrane, which are crucial for the removal of harmful compounds such as antibiotics from the intracellular environment. The efflux system can be divided into several categories, ranging from being compound-specific to universal pumps that allow bacteria to survive in the presence of antimicrobials. Inhibition of the activity of such pumps is of great importance in counteracting antibiotic resistance by reviving the efficacy of antibiotics. A previous study by Chovanová et al demonstrated efflux inhibition activity in essential oils extracted from three *Salvia* species against antibiotic-resistant *Staphylococcus epidermis*. Using fluorescent spectrophotometry, all three essential oils were found to reduce the efflux activity of *S. epidermis* upon exposure to essential oils.[10] The team further

revealed the increase in antibiotic susceptibility in *S. epidermis* after treatment with essential oil.[10] Soyingbe et al[3] showed similar activity of efflux inhibition by eucalyptus essential oil against respiratory tract infection bacteria such as *K. pneumoniae, S. aureus*, and *Moraxella catarrhalis*. The group also assessed the efflux inhibition activity using Rhodamine 6G accumulation assay, in which the treated cells exhibited increased accumulation of Rhodamine 6G resulting in a faulty bacterial efflux system.[3] In addition, De Morais Oliveira-Tintino et al also reported efflux inhibitory activity of essential oil from *Chenopodium ambrosioides* L. against drug-resistant *S. aureus*. The inhibitory activity was derived from the combined activity of antibiotic essential oil and ethidium bromide.[39] Interestingly, the essential oil alone showed no antibacterial activity against *S. aureus*, whereas essential oil combined with antibiotics significantly reduces the effective dose of antibiotics, resulting in a synergistic effect between essential oils and antibiotics.[39] Recently, Espinoza et al[40] demonstrated efflux inhibiting activity of heartwood essential oil in drug-resistant *S. aureus* with NorA multidrug efflux pump. The team performed ethidium bromide efflux assay against heartwood essential oil-treated *S. aureus* cells and found that treated cells had reduced efflux activity of ethidium bromide.[40]

The Missing Piece: Recent Approaches

The antimicrobial activities of essential oils due to membrane disruption and efflux inhibition have been well reported. Nevertheless, there is still a lack of knowledge of the underlying mechanism that confers these effects. Recent advances in genomic and proteomic methods, such as comparative gene expression, microarray, and comparative proteomic analysis, may provide the key missing piece to elucidate and explain the mode of action of essential oils.

Application of genomics to unravel the mode of action of antimicrobial effect in essential oils

The comparative analysis of gene expression between essential oil-treated and untreated cells is the main strategy used to assess genomic changes in the target pathogens induced by essential oils. Myszka et al investigated the ability of thyme essential oil to develop anti-quorum sensing and anti-biofilm formation against the opportunistic pathogen, *P. aeruginosa*. Their study found reduced quorum sensing and biofilm formation activity in thyme essential oil-treated *P. aeruginosa* using quorum sensing autoinducer and biofilm formation assay.[41] Further evaluation of flagella-related gene *flgA* that regulate quorum sensing activity showed reduce expression upon exposure to thyme essential oil.[41] Kovács et al compared the gene expression of peppermint essential oil-treated and untreated *Campylobacter jejuni* using comparative real-time polymerase chain reaction (PCR). A panel of genes related to pathogenic process, stress response, basic metabolism, and transcription regulation were screened and increased expression of oxidative stress response genes was found in essential oil-treated *C. jejuni*.[42] Lai et al investigated the effect of lavender essential oil against *E. coli* with comparative microarray analysis. Their study reported that lavender essential oil affects the phosphotransferase system and lipopolysaccharide biosynthesis pathway by upregulating genes involved in the pathway mentioned.[43] These revealed signs of membrane damage as increased expressions of phosphotransferase system and lipopolysaccharide biosynthesis genes indicated counter-response in bacterial membrane repair.

The application of proteomics to unravel the mode of action of antimicrobial effect in essential oils

The proteomic analysis involves an analysis of the proteome profile of untreated and essential oil-treated cells to identify and quantify differentially expressed proteins signals. Methods that allow such a comparison are 2D-SDS PAGE and LC-MS/MS. Kovács et al used 2D-SDS PAGE coupled with LC-MS/MS to compare the proteome of untreated and peppermint essential oil-treated *C. jejuni*. Their study showed an increased expression of oxidative stress-related proteins such as *dps, sodB*, and *katA* following treatment with peppermint essential oil.[42] In a similar approach, Barbosa et al investigated the effect of oregano essential oil on *Salmonella enteritidis*. A total of 15 oxidative stress-related proteins (*clpB, htpG, luxS*, toxic shock protein, and *usp*) were upregulated in oregano essential oil-treated *S. enteritidis* cells based on the assay developed using 2D-SDS PAGE coupled with LC-MS/MS.[44] Yang et al observed similar oxidative stress induction by comparing the proteome profile of untreated and cinnamon bark essential oil-treated clinical-relevant strain of *K. pneumoniae* cells using nano LC-MS/MS. The group has previously performed biochemical studies on the mode of action of cinnamon bark essential oil against the same clinically relevant strains of *K. pneumoniae*, revealing that essential oil disrupts bacterial membrane using zeta potential measurement, outer membrane permeability assay, and electron microscopy.[9] The follow-up study using nano LC-MS/MS showed an increase in the abundance of oxidative stress-sensitive proteins such as glycyl radical cofactor, catalase peroxidase and DNA mismatch repair protein, which indicates the presence of oxidative stress during cinnamon bark essential oil treatment.[45] Thus, they postulated that cinnamon bark essential oil induces oxidative stress that disrupts the bacterial membrane via lipid peroxidation. In a separate study, Yang et al investigated the mode of action of lavender essential oil on the proteome of *K. pneumoniae*. The team performed proteomic profiling between untreated and lavender essential oil-treated *K. pneumoniae* which has revealed an increase in the abundance of oxidative stress regulators such as NAD(P)H dehydrogenase (quinone) and autonomous glycyl radical cofactor while decreasing the abundance of oxidative stress-sensitive proteins, suggesting the presence of oxidative stress. The proteome profile also showed a drastic decrease in

membrane-related proteins (NADH-quinone oxidoreductase subunit B, outer membrane protein A and ATP synthase subunit C), and cytoplasmic proteins (mannitol-1-phosphate 5-dehydrogenase and D-erythrose-4-phosphate dehydrogenase), showed signs of membrane disruption.[5] Subsequent biochemical assays were carried out to validate the observation from the comparative analysis. For instance, scanning electron microscopy, zeta potential assay, outer membrane permeability assays, and intracellular material leakage assays were carried out to show membrane disruption whereas reactive oxygen species measurement and lipid peroxidation assays were performed to verify the presence of oxidative stress during lavender essential oil treatment. The assays mentioned previously revealed that lavender essential oil-treated cells have disrupted the bacterial membrane and increased reactive oxygen species, as well as lipid peroxidation activity. The team concluded that lavender essential oil disrupts the bacterial membrane by inducing oxidative stress and causing membrane disruption.[5] Both studies by Yang et al showed similarities in the mode of action of cinnamon bark and lavender essential oil in terms of proteomic profile, with both essential oil-treated proteomic profiles decreasing in membrane-related protein and cytoplasmic protein while increasing in oxidative stress response proteins. This indicated that both essential oils induced oxidative stress, which later resulted in lipid peroxidation of the bacterial membrane. Such a process disrupts the bacterial membrane, which leads to intracellular leakage and eventually kills the cell. Also, the presence of reactive oxygen species damages oxidative stress-sensitive materials such as protein and genetic material that would also be lethal to the treated cells.

Limitation and Future Strategies in Essential Oil Research

Essential oils serve as a good platform for the discovery of novel compounds that can be used in clinical settings. Nonetheless, the clinical approval milestone for utilization as an antimicrobial has not been met. It is mainly due to the complexity of the composition of each essential oil. Essential oils consist of a collection of different chemical compounds, ranging from terpenes and terpenoids to aromatic compounds. The essential oils of different plants also have a completely different composition and do not function the same way. For instance, cinnamon bark and lavender essential oil have completely different compositions as determined in previous studies.[5,46] Moreover, the same essential oil harvested from different regions can often result in different compositions, resulting in inconsistent therapeutic values. Besides, some of these compounds are often toxic in limited quantities and may not be completely appropriate for clinical use.

Future research should focus on the identification of novel compounds responsible for the mode of action of essential oils to minimize toxicity. This includes the assessment of antimicrobial efficacy, mode of action for elucidation using genomic and proteomic perspective, and biochemical assays. Antimicrobial efficacy includes the ability of the compound to exhibit antimicrobial activity on its own and the possibility that the compound may exhibit synergy with the combination of existing antibiotics. This will revive previously dormant antibiotics for combination therapy. Besides, a thorough toxicity study of potential substances, including human cell line toxicity tests and animal studies, should be performed. Human clinical trials will finally be feasible.

Furthermore, the delivery of the compound to the target site is another possibility of future clinical applications. Specialized delivery of nanoparticles to the target site will improve the effectiveness while reducing the toxicity of the compound itself.[47] The analysis by Patra et al[48] had consolidated evidence of improved therapeutic efficiency via targeted delivery while reducing toxicity. Thus, targeted and possibly personalized delivery is another future research prospect that can enhance the progress of current essential oil research.

To further study the mode of action of essential oils, both proteomic and genomic methodologies can be combined and analyzed. Proteomic analysis reveals the changes in the proteome profile, while genomic analysis reveals changes in the genetic transcript of essential oil-treated cells. This helps systems biology to understand whether essential oils may alter the gene expression that eventually affects protein expression or only the protein directly. Specialized software that allows the combinatory analysis of both the microarray and the proteomic profile of essential oil-treated cells can also be applied. This would facilitate the analysis and comparison of the proteomic and genomic profiles of the cells treated with different crude essential oils and the single compound-treated profiles from the same oil, thereby facilitating the elucidation of the mode of action of the compound.

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REFERENCES

1. Lewis K. Platforms for antibiotic discovery. *Nat Rev Drug Discov*. 2013;12:371-387.
2. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P T*. 2015;40:277-283.
3. Soyingbe O, Myeni C, Osunsanmi F, Lawal OA, Opoku A. Antimicrobial and efflux pumps inhibitory activities of Eucalyptus grandis essential oil against respiratory tract infectious bacteria. *J Med Plant Res*. 2015;9:343-348.
4. Yap PSX, Lim SHE, Hu CP, Yiap BC. Combination of essential oils and antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. *Phytomedicine*. 2013;20:710-713.

5. Yang SK, Yusoff K, Thomas W, et al. Lavender essential oil induces oxidative stress which modifies the bacterial membrane permeability of carbapenemase producing *Klebsiella pneumoniae*. *Sci Rep*. 2020;10:819.
6. Brochot A, Guilbot A, Haddioui L, Roques C. Antibacterial, antifungal, and antiviral effects of three essential oil blends. *Microbiologyopen*. 2017;6:e00459.
7. Yap PSX, Yang SK, Lai KS, Lim SHE. Essential oils: the ultimate solution to antimicrobial resistance in *Escherichia coli*. In: Samie A (ed.) Escherichia coli-*Recent Advances on Physiology, Pathogenesis and Biotechnology Applications*. InTech; 2017:299–313.
8. Schnitzler P, Schon K, Reichling J. Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. *Pharmazie*. 2001;56:343-347.
9. Yang SK, Yusoff K, Mai CW, et al. Additivity vs synergism: investigation of the additive interaction of cinnamon bark oil and meropenem in combinatory therapy. *Molecules*. 2017;22:1733.
10. Chovanova R, Mezovska J, Vaverkova S, Mikulasova M. The inhibition the Tet(K) efflux pump of tetracycline resistant *Staphylococcus epidermidis* by essential oils from three Salvia species. *Lett Appl Microbiol*. 2015;61:58-62.
11. Yap PS, Krishnan T, Yiap BC, Hu CP, Chan KG, Lim SHE. Membrane disruption and anti-quorum sensing effects of synergistic interaction between Lavandula angustifolia (lavender oil) in combination with antibiotic against plasmid-conferred multi-drug-resistant *Escherichia coli*. *J Appl Microbiol*. 2014; 116:1119-1128.
12. Hossain S, Heo H, De Silva BCJ, Wimalasena S, Pathirana H, Heo GJ. Antibacterial activity of essential oil from lavender *(Lavandula angustifolia)* against pet turtle-borne pathogenic bacteria. *Lab Anim Res*. 2017;33:195-201.
13. Firmino DF, Cavalcante TTA, Gomes GA, et al. Antibacterial and antibiofilm activities of *Cinnamomum* sp. essential oil and cinnamaldehyde: antimicrobial activities. *Sci World J*. 2018;2018:7405736.
14. Liang R, Xu S, Shoemaker CF, Li Y, Zhong F, Huang Q. Physical and antimicrobial properties of peppermint oil nanoemulsions. *J Agric Food Chem*. 2012;60:7548-7555.
15. Saeed S, Naim A, Tariq P. *In vitro* antibacterial activity of peppermint. *Pak J Bot*. 2006;38:869-872.
16. Hammer KA, Carson CF, Riley TV. Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on Candida albicans, *Candida glabrata* and *Saccharomyces cerevisiae*. *J Antimicrob Chemother*. 2004;53:1081-1085.
17. Powers CN, Osier JL, McFeeters RL, et al. Antifungal and cytotoxic activities of sixty commercially-available essential oils. *Molecules*. 2018;23:1549.
18. Hu F, Tu XF, Thakur K, et al. Comparison of antifungal activity of essential oils from different plants against three fungi. *Food Chem Toxicol*. 2019; 134:110821.
19. Schnitzler P, Koch C, Reichling J. Susceptibility of drug-resistant clinical herpes simplex virus type 1 strains to essential oils of ginger, thyme, hyssop, and sandalwood. *Antimicrob Agents Chemother*. 2007;51:1859-1862.
20. Vuko E, Rusak G, Dunkic V, et al. Inhibition of satellite RNA associated cucumber mosaic virus infection by essential oil of *Micromeria croatica* (Pers.) Schott. *Molecules*. 2019;24:1342.
21. Mulyaningsih S, Sporer F, Reichling J, Wink M. Antibacterial activity of essential oils from eucalyptus and of selected components against multidrug-resistant bacterial pathogens. *Pharm Biol*. 2011;49:893-899.
22. Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pac J Trop Biomed*. 2012;2:739-742.
23. Park JW, Wendt M, Heo GJ. Antimicrobial activity of essential oil of *Eucalyptus globulus* against fish pathogenic bacteria. *Lab Anim Res*. 2016;32: 87-90.
24. Gakuubi MM, Maina AW, Wagacha JM. Antifungal activity of essential oil of *Eucalyptus camaldulensis* Dehnh against selected *Fusarium* spp. *Int J Microbiol*. 2017;2017:8761610.
25. Schroder T, Gaskin S, Ross K, Whiley H. Antifungal activity of essential oils against fungi isolated from air. *Int J Occup Environ Health*. 2017;23:181-186.
26. Behmanesh F, Pasha H, Sefidgar AA, et al. Antifungal effect of lavender essential oil *(Lavandula angustifolia)* and clotrimazole on *Candida albicans*: an *in vitro* study. *Scientifica (Cairo)*. 2015;2015:261397.
27. D'Auria FD, Tecca M, Strippoli V, Salvatore G, Battinelli L, Mazzanti G. Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form. *Med Mycol*. 2005;43:391-396.
28. Puškárová A, Bučková M, Kraková L, Pangallo D, Kozics K. The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. *Sci Rep*. 2017;7:8211.
29. Schuhmacher A, Reichling J, Schnitzler P. Virucidal effect of peppermint oil on the enveloped viruses herpes simplex virus type 1 and type 2 *in vitro*. *Phytomedicine*. 2003;10:504-510.
30. Fu Y, Zu Y, Chen L, et al. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytother Res*. 2007;21:989-994.
31. Sienkiewicz M, Lysakowska M, Pastuszka M, Bienias W, Kowalczyk E. The potential of use basil and rosemary essential oils as effective antibacterial agents. *Molecules*. 2013;18:9334-9351.
32. Ojeda-Sana AM, Van Baren CM, Elechosa MA, Juárez MA, Moreno S. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. *Food Control*. 2013;31:189-195.
33. Moghtader M, Salari H, Farahmand A. Evaluation of the antifungal effects of rosemary oil and comparison with synthetic borneol and fungicide on the growth of *Aspergillus flavus*. *J Ecol Nat Environ*. 2011;3:210-214.
34. Cox SD, Mann CM, Markham JL, Gustafson JE, Warmington JR, Wyllie SG. Determining the antimicrobial actions of tea tree oil. *Molecules*. 2001;6:87-91.
35. Lee CJ, Chen LW, Chen LG, et al. Correlations of the components of tea tree oil with its antibacterial effects and skin irritation. *J Food Drug Anal*. 2013;21:169-176.
36. Cassella S, Cassella JP, Smith I. Synergistic antifungal activity of tea tree *(Melaleuca alternifolia)* and lavender *(Lavandula angustifolia)* essential oils against dermatophyte infection. *Int J Aromather*. 2002;12:2-15.
37. Garozzo A, Timpanaro R, Bisignano B, Furneri PM, Bisignano G, Castro A. *In vitro* antiviral activity of *Melaleuca alternifolia* essential oil. *Lett Appl Microbiol*. 2009;49:806-808.
38. Zhang J, Ye KP, Zhang X, Pan DD, Sun YY, Cao JX. Antibacterial activity and mechanism of action of black pepper essential oil on meat-borne *Escherichia coli*. *Front Microbiol*. 2017;7:2094.
39. De Morais Oliveira-Tintino CD, Tintino SR, Limaverde PW, et al. Inhibition of the essential oil from *Chenopodium ambrosioides* L. and α-terpinene on the NorA efflux-pump of *Staphylococcus aureus*. *Food Chem*. 2018;262:72-77.
40. Espinoza J, Urzúa A, Sanhueza L, et al. Essential oil, extracts, and sesquiterpenes obtained from the heartwood of *pilgerodendron uviferum* act as potential inhibitors of the *Staphylococcus aureus* NorA multidrug efflux pump. *Front Microbiol*. 2019;10:337.
41. Myszka K, Schmidt MT, Majcher M, Juzwa W, Olkowicz M, Czaczyk K. Inhibition of *quorum sensing*-related biofilm of *Pseudomonas fluorescens* KM121 by *Thymus vulgare* essential oil and its major bioactive compounds. *Int Biodeter Biodegr*. 2016;114:252-259.
42. Kovacs JK, Felso P, Horvath G, et al. Stress response and virulence potential modulating effect of peppermint essential oil in *Campylobacter jejuni*. *Biomed Res Int*. 2019;2019:2971741.
43. Lai PJ, Ng EV, Yang SK, et al. Transcriptomic analysis of multi-drug resistant Escherichia coli k-12 strain in response to *Lavandula angustifolia* essential oil. *Biotech3*. 2020;10:313.
44. Barbosa LN, Alves FCB, Andrade BFMT, et al. Proteomic analysis and antibacterial resistance mechanisms of *Salmonella enteritidis* submitted to the inhibitory effect of *Origanum vulgare* essential oil, thymol and carvacrol. *J Proteom*. 2020;214:103625.
45. Yang SK, Yusoff K, Ajat M, et al. Disruption of KPC-producing Klebsiella pneumoniae membrane via induction of oxidative stress by cinnamon bark *(Cinnamomum verum* J. Presl) essential oil. *PLoS ONE*. 2019;14:e0214326.
46. Yap PS, Krishnan T, Chan KG, Lim SHE. Antibacterial mode of action of *Cinnamomum verum* bark essential oil, alone and in combination with piperacillin, against a multi-drug-resistant *Escherichia coli* strain. *J Microbiol Biotechnol*. 2015;25:1299-1306.
47. Moo CL, Yang SK, Yusoff K, et al. Mechanisms of antimicrobial resistance (AMR) and alternative approaches to overcome AMR. *Curr Drug Discov Technol*. 2019;16. doi:10.2174/1570163816666190304122219.
48. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotech*. 2018;16:71.